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**ELECTROPHORESIS PATTERN OF SERUM FROM MICE EXPOSED
TO DIFFERENT CONCENTRATIONS OF SULFUR DIOXIDE**

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ABSTRACT

Three day old mice were continuously exposed to sulphur dioxide concentrations at 0ppm, 0.05ppm, 0.15ppm and 1ppm for eight weeks. At the end of the experiment, blood samples were collected and centrifuged for electrophoresis studies of the serum in 5 percent acrylamide gel. The length of bands of different serum proteins from the SO₂ exposed mice was at a variance as compared with the length of bands from the control exposed mice and alpha-1 band seems to be missing from the serum of SO₂ exposed mice.

Sulphur dioxide (herein after referred to as SO₂) is a non-flammable, non-explosive colorless gas that most people can taste at concentrations from 0.3 to 1ppm in air. At concentrations above 3ppm the gas has a pungent, irritating odour. The current scientific literature indicates that for the most part, the effects of oxides of sulphur on health are related to the irritation of the respiratory system (1-5). Such injury may be temporary or permanent. Laboratory observations of respiratory irritating gases suggest that most individuals will show a response to SO₂ at concentrations of 5ppm. At concentrations of 1 to 2ppm an effect can be detected only in certain sensitive individuals (1) and on occasions, exposure to 5 and 10ppm has produced severe bronchospasm in such person. Exposure of SO₂ at 1 to 15ppm decreased the average weight of body, liver, kidneys, heart, brain and spleen and at the same time increased the RBC (Red Blood Corpuscles), HCT (Hematocrite) and HGB (Hemoglobin) in laboratory mice (6). Since sulphur is an important component of some of the amino acids and proteins, it is reasonable to assume that sulphur in the form of SO₂ may alter the protein pattern in serum. Therefore an experiment was conducted to observe the changes in the electrophoresis pattern of mice as a result of exposure to SO₂ at 0, 0.05, 0.15 and 1.0ppm levels.

Materials and Methods: Exposure of animals sulphur dioxide: Four environmental chambers (one each for 0ppm, 0.05ppm, 0.15ppm and 1.0ppm SO₂) were placed in an air conditioned room in which the average temperature and humidity were 74± 2°F and 54± 2

respectively. One litter of three day old mice along with the mothers was placed in plastic cages with wire covers. Three litters in cages were placed in each environmental chamber with gases on at a flow of 120 ml/minute all the times except for 5 hours a week. This time was used to clean, change and sterilize the cages and chambers. The mice were given 5 minutes of fresh air every day. The food was a special sterile commercial product (lab blox) for mice and like the water was available to mice at all times. The litters were weaned after the first two weeks of exposure and separated by the sexes. At the end of 8 weeks one mice from each chamber was sacrificed and blood samples for serum collected. All specimens of serum were clear and free of hemolysis.

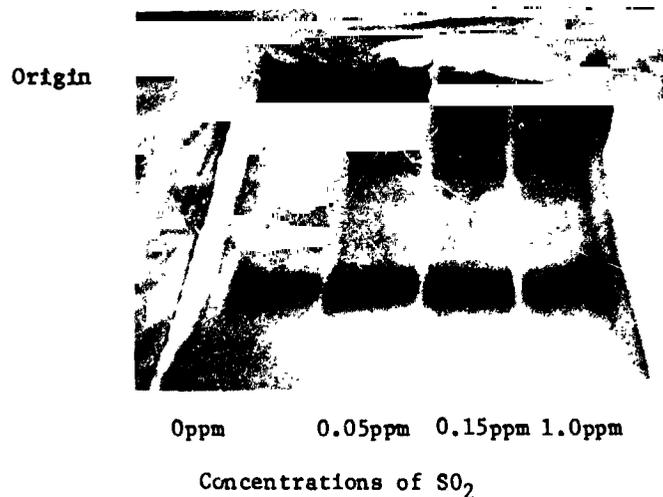
Preparation of the Gel: The electrophoresis gel was prepared with 5 percent acrylamide gel as described by Peacock, Bunting and Queen (7). The gel was prepared before run from the following refrigerated stock solutions: (A) Acrylamide, 20 percent: acrylamide 190 grams, N-N methylenebisacrylamide 10 grams, dissolved in two liters of water; (B) DMAPN-: (dimethylamino propionitrile) 16 ml diluted to 250 ml; (C) ammonium persulphate: 1.6 percent in water, prepared fresh. To prepare the gel, 40 ml of acrylamide stock (Reagent A), 84 ml of water, 10 ml of DMAPN-buffer (Reagent B) and 16 ml of 10X buffer (described below) were mixed and shaken well and brought to room temperature by running tap water over it. To this 10 ml of ammonium persulphate (Reagent C) was added. The whole mixture was mixed well and poured into the cell just before the run. The 10X buffer contained 110 grams boric acid, 18.6 grams disodium ethylenediamine tetracetate, 216 grams of tris (hydroxymethyl) amino methane and 2 liter of water.

The voltage was applied for 30 minutes before the addition of samples. The serum used was obtained from the mice blood at the end of eight weeks exposure. All the specimens were clear and free of hemolysis. A four place slot former was used. Water at 0-2°C (for cooling) was circulated through the cell throughout the run. A potential difference of 200 volts was applied. The experiment was run for 3 hours. The gel was stained for 1 hour in 1 percent amido black 10 B in 7.5 percent acetic acid and destained overnight in 7.5 percent acetic acid circulated through at charcoal bath. After destaining was complete, the gel was photographed with polaroid 70 SX camera.

Results and Comments: The photograph of the electrophoresis pattern of mice serum is shown in the figure. The migration distance of the serum in the gel for 0, 0.05, 0.15 and 1ppm SO₂ exposure was 11.5, 11.3, 11.5 and 11.3 cms. respectively up to the prealbumin line. The serum from control exposed mice seems to have all the characteristics serum proteins e.g. gamma, beta, alpha-2, alpha-1, albumin and prealbumin. These are shown from

top of the figure to the bottom respectively. The alpha-2 band in the serum from the control and SO₂ exposed mice is further split into two bands. The most significant observation of the experiment is that alpha-1 band is altogether missing from the serum of 0.05, 0.15 and 1ppm SO₂ exposed mice. The

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Photograph of the electrophoresis verticle gel showing the pattern of serum proteins from mice exposed to various concentrations of sulphur dioxide for eight weeks. Serum from 0ppm SO₂ exposed mice show all the serum proteins. Alpha-1 proteins is absent from the serum of 0.05, 0.15 and 1.0ppm SO₂ exposed mice. The dista ce from the origin to the leading edge of the albumin is 11.5 cms.

deficiency or absence of alpha-1 globulin may be due to the deficiency of alpha-1 antitrypsin as a result of SO₂ stress. Alpha-1 antitrypsin deficiency is also associated with pulmonary diseases like emphysema and hepatic cirrhosis (8).

Work on the effects of SO₂ on the vital organs like lungs is in progress and will be reported in another manuscript.

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